EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 4
308-313
Year: 2016

COMPARATIVE STUDY OF SUREPATH LIQUID BASED CYTOLOGY WITH CONVENTIONAL PAP SMEAR IN SCREENING OF CERVICAL CANCER

Ankita Goel*, Manjit S Bal., Parmjit Kaur and Anudeep Gill

[1]Senior Resident, Department of Pathology, Government Medical College, Patiala, India.
[2]Professor, Department of Pathology, Government Medical College, Patiala, India
[3]Professor, Department of Obstetrics and Gynaecology, Government Medical College, Patiala, India.
[4]Senior resident, Department of Pathology, Government Medical College, Patiala, India.

***Author for Correspondence: Dr. Ankita Goel**
Senior Resident, Department of Pathology, Government Medical College, Patiala, India.

Article Received on 24/01/2016 **Article Revised on 15/02/2016** **Article Accepted on 07/03/2016**

ABSTRACT
Background: Pap smear is a tool for screening "invisible" cervical abnormalities under light microscopy. Cervical cancers are preceded by precancerous lesions that can be detected by Pap test. The conventional Pap smear (CPS) technique is sometimes difficult to interpret due to overlapping cells, blood or inflammation. Liquid Based Cytology (LBC) enables cells to be suspended in a monolayer, improving specimen adequacy. Aim: To analyze satisfactory and unsatisfactory rates of LBC with CPS and compare the efficacy of LBC and CPS in the detection of normal smear, inflammation and epithelial cell abnormality. Materials and Methods: 300 cell samples were collected using cervex brush by split sample method and received in the Department of Pathology, Govt. Medical College, Patiala. LBC vial was processed by SurePath LBC. In each case, two smears were compared for the percentage of abnormalities detected. Statistical analysis: Pearson chi square test was used to analyze the data. P value of 0.05 or less was considered statistically significant. Results: Percentage of unsatisfactory samples decreased from 9.3% (CPS) to 0.3 % (LBC). The morphology of cells in samples showing excess of inflammation or excess blood was not obscured in LBC smears. The detection of epithelial cell abnormalities was increased from 13% to 21% (p value .018). Conclusions: LBC is highly effective in diagnosing precancerous and cancerous lesions of cervix in comparison to CPS by reducing the number of unsatisfactory specimens and improving cell details.

KEYWORDS: Liquid based cytology (LBC), conventional pap smear (CPS), epithelial cell abnormality, cervical cancer**.**

INTRODUCTION
Cervical cancers and precancerous lesions can be detected on cervical cytologic examination (Pap test).[1] Conventional Pap smears (CPS) is sometimes difficult to interpret due to uneven cell distribution, overlapping cells, blood or inflammation.[2] Such unsatisfactory smears require retesting. This increases the costs and entails more anxiety for the women.[3]

Liquid Based Cytology (LBC) was developed in the 1960's as an alternative to CPS. In LBC, samples are centrifuged to disperse the cells, cellular debris, blood or mucus is removed and cells are suspended in a monolayer, thus detection of precursor lesions is improved.[4][5] The residual sample can be used for HPV DNA and immunocytochemistry.[4]The present study was undertaken to compare the utility of CPS and LBC smears in diagnosing cervical cancer and its precursors.

MATERIALS AND METHODS
The study comprised of simultaneous Conventional Pap Smear and Liquid Based Cytology smear in 300 patients. All married women visiting Gynaecology Outpatient Department with various gynecological complaints were screened for early detection of cervical cancer. Cell samples were collected using split sample method in which cervical cells were first transferred to a conventional slide, after that same brush head was detached and suspended in LBC vial containing preservative fluid. Cervical smears and vial for LBC were received, processed and stained in the Department of Pathology, Govt. Medical College, Patiala. The fixed CPS was subjected to staining according to Papanicolaou's method. LBC smears were stained in SurePaths Prepstain™ Slide Processor. Cervical samples were compared for multiple parameters like morphology of various cells and U/S rates of two methods for detection of epithelial abnormalities as per the Bethesda system (TBS) 2001. The study was approved by the

Institutional Review Committee and an oral informed consent was taken from every patient.

Statistical analyses:-Data was analyzed using the statistical package SPSS version 15 for MS- Windows (SPSS Inc., Chicago, IL). Pearson chi square test is used to analyze the data and p-value is calculated wherever required. P-value of 0.05 or less was considered statistically significant.

RESULTS

Unsatisfactory Smears: - It was found that the percentage of unsatisfactory samples decreased from 9.3% (CPS) to 0.3 % in LBC. (Table 1)

Main cause of unsatisfactory samples in LBC was low cellularity. Whereas in CPS, the main cause of unsatisfactory smear was low cellularity followed by obscuration by excess blood.

The morphology of cells in samples showing excess of inflammatory cells or excess blood was not obscured in LBC smears. According to this study, 6/7 cases (85.7%) cases of SCC reported in LBC were unsatisfactory due to blood in CPS. 1/2 (50%) cases of HSIL were unsatisfactory due to blood and 1/2 (50%) were unsatisfactory due to low cellularity in CPS. (Table 2)

Split sample (CPS and LBC samples from the same patient) were reported on cytology according to TBS 2001. Break-up of "split samples" reported as per TBS 2001 is given in (Table 3).

Epithelial abnormalities detected in 300 split Pap samples (Table 4)

Detection rate of LSIL increased from 3.3% in CPS to 3.7% in LBC. Recognition of HSIL increased from 3.7% to 6.0%.Identification of SCC was increased from 1.7% to 4.6%. Tumour diathesis important for diagnosis of SCC was less evident on LBC samples. However, there was less obscuration of morphology of cells due to excess blood and inflammation in LBC smear, resulting increased detection rate of SCC. Glandular cells abnormalities were more commonly seen in LBC samples, 1.0% in CPS and 2.0% in LBC.

Comparison of Morphological characteristics in CPS versus LBC

Atypical squamous cell- of undetermined significance (ASCUS) and Low grade squamous intraepithelial lesion(LSIL) - Smears reported as LSIL showed singly scattered and groups of intermediate sized squamous cells with nuclear enlargement (>3 times area of normal intermediate nucleus), slight increase in nuclear: cytoplasmic (N:C) ratio, coarse chromatin and slightly irregular nuclear membranes. In addition, koilocytic atypia was noted in all, defined by perinuclear halo with peripheral rim of densely stained cytoplasm associated with nuclear changes. Prominent bi and multinucleation was also noted. These changes were appreciated both in CPS and LBC samples, however nuclear details were much clearer in LBC smears and it was easier to appreciate koilocytosis in LBC smears. Cases which fall short of LSIL but had changes more than reactive atypia were reported as ASCUS (Figure1a, b).

Atypical squamous cells – cannot exclude HSIL (ASC-H) - Cases reported as ASC-H show almost similar features in CPS and LBC. There were scattered as well as small fragments of less than 10 small cells with high N/C Ratios (Atypical/Immature metaplastic cells) in the background of LSIL. In Liquid-Based preparations, ASC-H cells appeared quite small, with nuclei that were only two to three time the size of the neutrophil nuclei (Figure 1c, d).

High grade squamous intraepithelial lesion (HSIL) - Both CPS and LBC smears showed cytological feature of HSIL. There were scattered as well as groups of abnormal cells. These cells were of parabasal cell size and had high N: C ratio, coarse chromatin and inconspicuous nucleoli. The cell size appeared much smaller in LBC and nuclear chromatin was not as hyperchromatic as seen in CPS. One case showed a micro-biopsies/ hyperchromatic crowded cell groups (HCGs) in LBC sample, with loss of polarity associated with cellular abnormalities (Figure 2a, b). Another feature which was seen in both cases was presence of small dyskeratotic cells/ marker cells, which were conspicuous in LBC smears.

Squamous cell carcinoma (SCC) - Cases of SCC showed almost similar features in CPS and LBC samples. Tumour diathesis in LBC is relatively difficult to pick and appear as some fibrin rich tangles with entrapped inflammatory cells and tumour cells. CPS samples showed excess of blood obscuring the morphology of tumour cells, which was clearer in LBC samples. One case of keratinizing squamous cell carcinoma (K-SCC) was reported. The malignant cells showed marked pleomorphism. Many spindle cells with dense orangeophilic cytoplasm were seen. Occasional keratin pearl was also noted. Tumour diathesis was present but less than that seen in nonkeratinizing squamous cell carcinomas (NK-SCC). The corresponding CPS of split sample was reported as unsatisfactory because the cell details were obscured by blood (Figure 2c, d).

Atypical Glandular Cells- AGC-NOS and AGC-FN were diagnosed on LBC as well as CPS. Atypical glandular cell: not otherwise specified (AGC-NOS) - Cells occurred in sheets and strips with some crowding and nuclear overlap. Cells had mildly increased N: C ratio (upto 3 times), mild pleomorphism and hyperchromasia with abundant cytoplasm. Distinct cell borders were discernible (Figure 3a, b).

Atypical glandular cells, favour neoplastic (AGC-FN): Abnormal cells occurred in sheets and strips with nuclear

crowding and overlap. Rare cell groups showed rosetts and feathering. Cells possessed high N:C ratio and hyperchromasia with indistinct cell borders. In Liquid-based preparations, groups were three dimensional and thick with layers of cells obscuring central nuclear detail (Figure 3c, d).

Other morphological features and differences observed - The conventional smears showed clumping of cells whereas the LBC smears were well-spread and it was easier to see the morphology of individual cells. SurePath LBC samples were characterized by the presence of microbiopsies/ hyperchromatic crowded cell groups (HCGs), which can represent both normal and abnormal cervical cells. These cell groups were difficult to evaluate at times, needing training/ experience. The drying/ smearing artifacts were not seen in the LBC smears but many CPS showed some drying/ smearing artifacts.

Most patients of LSIL (45.5%) presented in the age group of 18-30 year, HSIL (38.9%) in 46-60 years and SCC (85.6%) in 31-60 years. It was observed that as the age advances, there was sequential progression in the development of LSIL to HSIL to invasive carcinoma and is statistically significant with p value <0.001.

Another established risk factor for cervical cancer is high parity. According to this study, maximum cases of LSIL were reported in uniparous women (45.4%). Almost all cases of HSIL (83.3%) were seen in multiparous women with parity 2 or more. All cases of SCC were reported in multiparous women. Thus the frequency of both SIL and cervical cancer showed a progressive rise with increasing parity is statistically significant with p value <0.001.

History of oral contraceptive (OCP) use was present in 31 women. Of which only 3/8 (16.7%) patients had HSIL and 3/14 (21.4%) patients had SCC; showing a poor association (p=0.989) between oral contraceptive use and cervical cancer.

TABLES AND FIGURES

Table -I Type of smears in split samples (CPS AND LBC)

TYPE OF SMEARS	CPS		LBC	
	No. of Cases	Percentage	No. of Cases	Percentage
Satisfactory	272	90.7	299	99.7
Unsatisfactory	28	9.3	1	0.3
Total	300	100.0	300	100.0
P value		<0.001		

Table –II. Comparison of causes of unsatisfactory smears in CPS with diagnosis of LBC

Causes of unsatisfactory smear in CPS	Diagnosis LBC								
	ANS (n=4)	Infla-mmatory (n=9)	ASCUS (n=9)	ASC-H (n=1)	HSIL (n=2)	SCC (n=7)	AGC (n=3)	U/S (n=1)	Total
Low cellularity	4 (100%)	6 (66.7%)	-	-	1 (50%)	-	1 (33.3%)	1 (100%)	13
Blood	-	1 (11.1%)	1 (100%)	1 (100%)	1 (50%)	6 (85.7%)	2 (66.7%)	-	12
Blood and inflammation	-	-	-	-	-	1 (14.3%)	-	-	1
Inflammation	-	2 (22.2%)	-	-	-	-	-	-	2
Total	4	9	1	1	2	7	3	1	28
P value	0.018								

Table III. Comparison of results of CPS and LBC

Results	CPS		LBC	
	No. of Cases	%age	No. of Cases	%age
Adequate normal smear	51	17.0	69	23.0
Inflammatory smear	179	59.6	167	55.7
Epithelial cell abnormality	42	14.0	63	21.0
Unsatisfactory	28	9.3	1	0.3
Total	300	100.0	300	100.0
P- value		<0.001		

Table IV. Comparison of epithelial cell abnormalities of CPS and LBC

Diagnosis	CPS		LBC	
	No. of Cases	%age	No. of Cases	%age
ASCUS	7	2.3	6	2.0
LSIL	10	3.3	11	3.7
ASC-H	6	2	8	2.7
HSIL	11	3.7	18	6.0
NKSCC	5	1.7	13	4.3
K-SCC	0	0.0	1	0.3
AGC-NOS	2	0.7	3	1
AGC-FN	1	0.3	2	0.6
ASC-H+ AGC-NOS	0	0.0	1	0.3
P-value		<0.001		

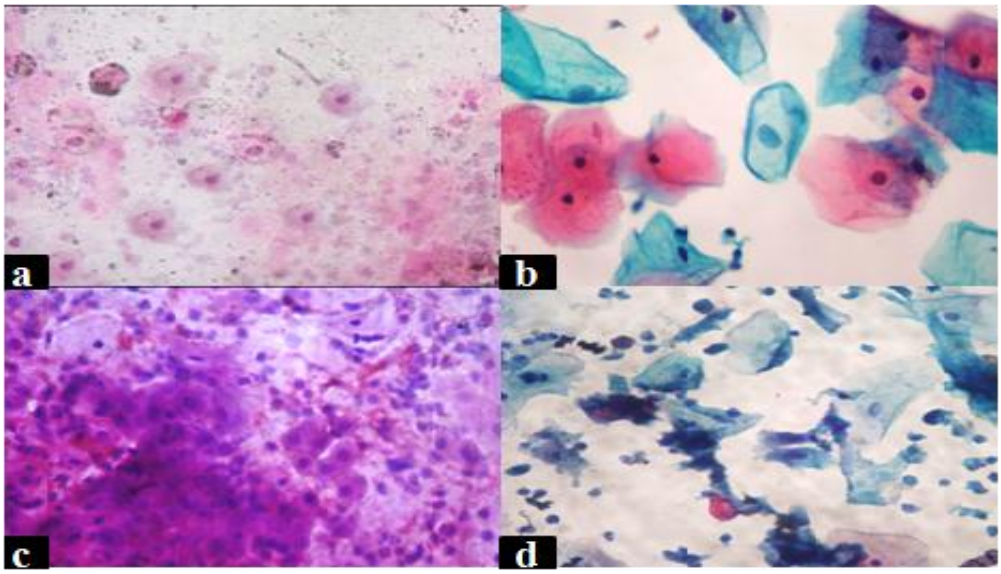

Figure 1

1a, b- Photomicrograph of LSIL showing perinuclear halo and mild nucleomegaly in CPS and LBC (PAP X 400).
1c- Photomicrograph of ASC-H showing small fragment of atypical metaplastic cell in CPS (PAP x 400)
1d- Photomicrograph of ASC-H showing scattered atypical metaplastic cells in the background of LSIL in LBC (PAP x 400)

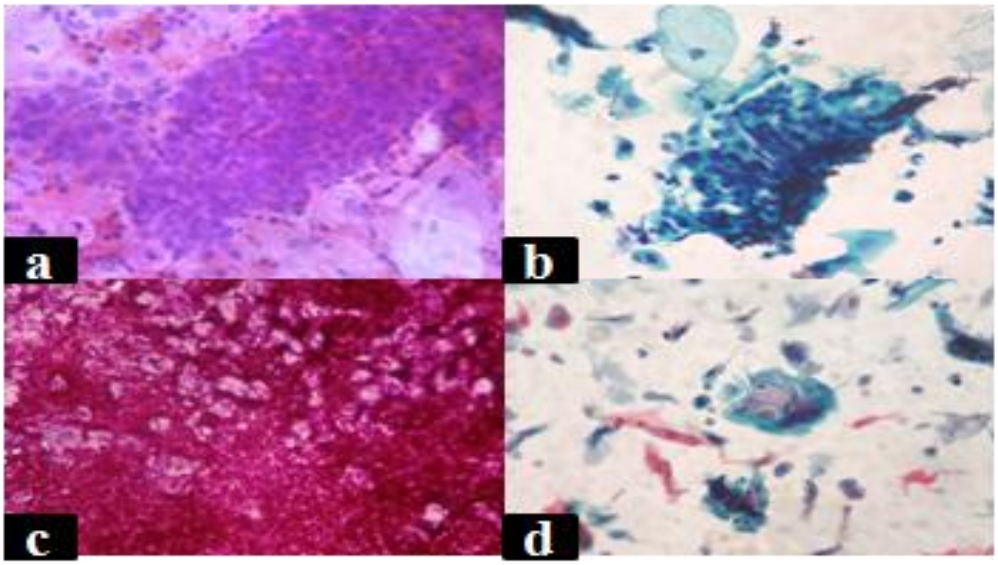

Figure 2

2a,b- Photomicrograph of HSIL showing hyperchromatic cell group composed of parabasal cells exhibiting nucleomegaly and overlapping nuclei in CPS and LBC (PAP X 400)

2c- Photomicrograph of CPS showing cellular details obscured by excess blood (PAP x 100)
2d- Photomicrograph of corresponding LBC smear showing keratin pearl of keratinizing squamous cell carcinoma (PAP x 400)

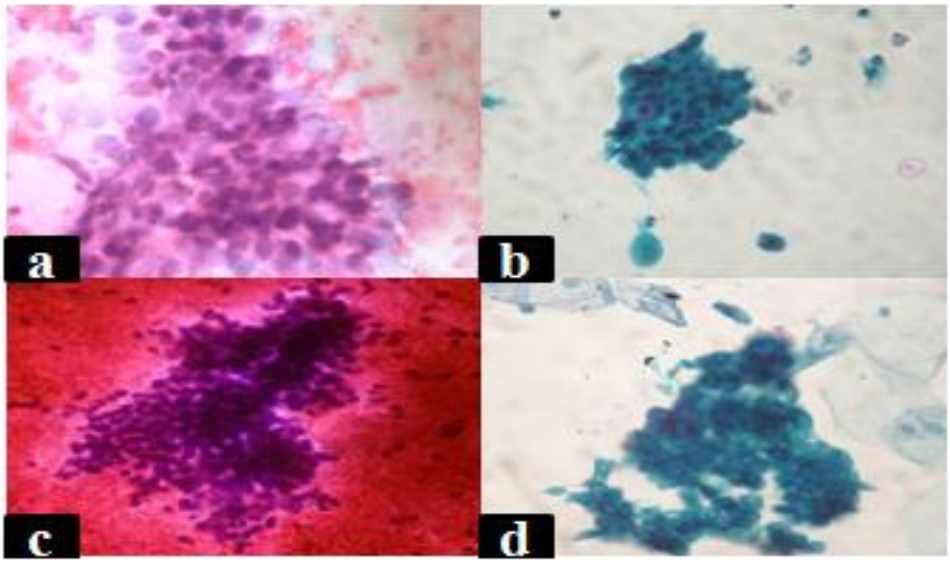

Figure3

3a,b- Photomicrograph of atypical endocervical cells not otherwise specified showing atypical endocervical cells exhibiting mild nucleomegaly, abundant cytoplasm with distinct cell borders in CPS and LBC (PAP x 400)
3c,d- Photomicrograph of atypical endocervical cells, favour neoplastic showing atypical endocervical cells exhibiting nuclear crowding, overlapping, hyperchromatism, feathering with indistinct cell borders In CPS and LBC (PAP x 400).

DISCUSSION

The Papanicolaou smear has been utilized for cervical cancer screening for more than 50 years. Despite being credited with a 70% reduction in mortality for cervical cancer, the false negative rate is still a cause for concern. It is widely acknowledged that two third of the overall false negative rate can be attributed to sampling Errors LBC is an alternate technique for processing the cervical sample collected. Most western countries have switched over from CPS to LBC, even though the sensitivity and specificity is almost similar in various comparison studies.

This study was conducted to evaluate the performance of LBC in low resource setting and to study the differences in morphology of various lesions in CPS and LBC samples. According to present study there is significant decrease in unsatisfactory Pap smear from 9.3% (CPS) to 0.3% (LBC). The reason for this may be (1) obscuration of morphology by dense inflammation/haemorrhagic background, (2) loss of cells while transferring the material on to glass slides, (3) inconsistent preservation (which depends largely on the persons collecting the smears) and (4) prolonged screening time in CPS. On the contrary LBC is semi -automated and offers clarity of staining, improved sample processing and small area of smear to be screened.

There are many studies which have documented similar unsatisfactory rate on both types of preparations. Sherwani et al[6] reported decrease in unsatisfactory smears from 68.1% in CPS to 16.9% in LBC. Another study by Nandini et al[4] showed 9% unsatisfactory smears in CPS and 1% in LBC.

The most common reason for unsatisfactory smear was low cellularity 4.3% in CPS and 0.3% in LBC. Second most common reason was excess blood (4%) in CPS. There was no inadequate LBC sample due only to excess blood or obscuration by polymorphs/ mucus or other technical artifacts. This indicates that the most common cause of unsatisfactory smear in LBC samples is technical/ sample taking. The causes of unsatisfactory smears in LBC samples and CPS are consistent with the studies of Nandini et al[4], Sherwani et al[6] and Siebers et al.[7]

According to this study, the rate of epithelial cell abnormalities was 14% in CPS and 21% in LBC. The detection of LSIL in CPS was 3.3%, ASC-H (2%), HSIL (3.7%) and SCC(1.7%) and detection of LSIL in LBC was 3.7%, ASC-H (2.7%), HSIL (6%) and SCC(4.6%%) The detection rate was higher in LBC samples, which may be explained by a better collection device and better morphology in LBC. The results are in congruence to the studies of Zhu et al[2] and Sherwani et al.[6]

Sherwani et al[6] in their study of comparison of conventional pap smear and liquid based cytology in 2007 found an increase in detection of LSIL from 10.6 in CPS to 18.2% in CPS, HSIL from 0.6% in CPS to 4.3% and SCC was same 3.7% in both CPS and LBC. Zhu et

al found increase in LSIL detection rate from 29% in CPS to 32% in LBC and HSIL detection rate from 29% in CPS to 42% in LBC.

Morphologically, the epithelial cell abnormalities especially HSIL lesions appear different in LBC as compared to HSIL in CPS. In LBC samples, koilocytes were detected more easily and nuclear features were better preserved due to lack of obscuring background and better preservation of cells. HSIL cells in LBC samples appeared smaller in size. Nuclear abnormalities in the form of nuclear membrane irregularity and chromatin condensation were better seen on LBC samples. In SCC, both type of preparations showed similar morphology but tumour diathesis important for diagnosis of SCC was sometimes less evident on LBC samples. However, there was less obscuration due to inflammation or blood in the LBC samples which obscured morphology of tumour cells in CPS. The morphology of various epithelial cell abnormalities detected were in accordance with the findings of Nandini et al[4] and Sherwani et al.[6]

Studies conducted by Burnley et al[8] and Park [9] found an increase in detection of glandular cell abnormality from 2.2% in CPS to 3.9% in LBC and 0.03% in CPS to 0.09% in LBC respectively. These findings are similar to our study showing increase in detection of glandular cell abnormality from 1% in CPS to 1.9% in LBC. The improvement in detection of endocervical lesions is probably related to a combination of factors including: more effective sampling of endocervical canal due to change of sampling device from spatula to cytobrush, more representative transfer of cells from the sampling device to the liquid medium used for processing and improved morphological presentation of endocervical abnormalities particularly evident with SurePath

According to this study, maximum cases of LSIL were reported in uniparous women (45.4 %). Almost all cases of HSIL (83.3%) were seen in multiparous women with parity 2 or more. All cases of SCC were reported in multiparous women. Thus the frequency of both SIL and cervical cancer showed a progressive rise with increasing parity. High parity usually means frequent coitus during many years, starting at younger age. This correlates well with the study of Bhojani et al.[10] Hence multiparty is a significant risk factor for cervical cancer.

LIMITATION

The lower incidence of LSIL in our study as compared to HSIL and SCC might be attributable to selection bias since both CPS and LBC smears were prepared mostly in symptomatic women because of relatively high cost of LBC.

CONCLUSION

LBC in comparison to CPS improves the effectiveness of cervical cancer screening by increasing the detection of neoplastic and pre-neoplastic disease while simultaneously decreasing over diagnosis of benign process and offers higher accuracy.

REFERENCES

1. Nasreen M, Ahamad MSU, Bhattacharjee M, Rahman MZ. Evaluation of Conventional Pap Test for Cervical Intraepithelial lesions and Cancer in a Tertiary Hospital of Bangladesh. ChattagramMaa-O-Shishu Hospital Medical College Journal., 2013; 12: 1-6.
2. Zhu J, Norman I, Elfgren K, Gaberi V, Hagmar B, Hjerpe A et al. A comparison of liquid-based cytology and Pap smear as a screening method for cervical cancer. Oncol Rep., 2007; 18: 157-60.
3. Kirschner B, Simonsen K, Junge J. Comparison of conventional Papanicolaou smear and SurePath liquid-based cytology in the Copenhagen population screening programme for cervical cancer. Cytopathology., 2006; 17: 187-94.
4. Nandini NM, Nandish SM, Pallavi P, Akshatha SK, Chandrashekhar AP, Anjali S et al. manual liquid based cytology in primary screening for cervical cancer- a cost effective preposition for resource settings. Asian pacific J cancer prev., 2012; 13: 3645-51.
5. Denton KJ. Liquid based cytology in cervical cancer screening. BMJ., 2007; 335: 1–2.
6. Sherwani RK, Khan T, Akhtar K, Zeba A, Siddiqui FA, Rahman K et al. Conventional Pap smear and liquid based cytology for cervical cancer screening - a comparative study. J Cytol., 2007; 24: 167-72.
7. Siebers AG, Klinkhamer PJ, Vedder JE, Arbyn M, Bulten J. Causes and relevance of unsatisfactory and satisfactory but limited smears of liquid-based compared with conventional cervical cytology. Arch Pathol Lab Med., 2012; 136: 76-83.
8. Burnley C, Dudding N, Parker M, Parsons P, Whitaker CJ, Young W. Glandular neoplasia and borderline endocervical reporting rates before and after conversion to the SurePath(TM) liquid-based cytology (LBC) system. Diagn Cytopathol., 2011; 39: 869-74.
9. Park J, Jung EH, Kim C, Choi YH. Direct-to-vial comparison of a new liquid-based cytology system, liqui-PREP versus the conventional pap smear. DiagnCytopathol., 2007; 35: 488-92.
10. Bhojani KR and Garg R. Cytopathological study of cervical smears and correlation of findings with risk. Inj J Biol Med Res., 2011; 2: 757-61.